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Mycorrhizal roles in plant growth, gas exchange, root morphology, and nutrient uptake of walnuts

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Abstract: Walnut, an important oil fruit tree, is dependent on arbuscular mycorrhizas, while mycorrhizal roles and efficient mycorrhizal fungus in walnuts are unknown. This study was conducted to evaluate the effect of five arbuscular mycorrhizal fungi (AMF) species, including *Acaulospora scrobiculata*, *Diversispora spurca*, *Glomus etunicatum*, *G. mosseae*, and *G. versiforme* on plant growth, leaf gas exchange, root morphology, and root nutrient contents of walnut (*Juglans regia* L. Liaohe 1) seedlings. Three months of AMF inoculations later, root mycorrhizal colonisation achieved 47.0% to 76.4%. AMF treatments increased plant growth performance, dependent on AMF species. AMF-inoculated plants with *D. spurca*, *G. etunicatum*, and *G. mosseae* showed higher root length, projected area, surface area, and volume than non-AMF plants. Except for *G. versiforme*, the other four AMF treatments almost significantly increased leaf photosynthesis rate, transpiration rate, and stomatal conductivity, while reduced intercellular CO_2 concentrations and leaf temperature. AMF affected root nutrient contents, dependent on AMF and mineral nutrient species. These results, thereby, concluded that AMF had a positive role in walnuts, dependent on AMF species, and *D. spurca* was the best mycorrhizal fungus for walnut. Such results provide the potential possibility of a developing consortium of AMF in walnut cultivation management.

Keywords: endophytic fungi; mineral nutrients; mycorrhizal symbiosis; rhizosphere; root colonisation

Arbuscular mycorrhizal fungi (AMF) are one of the most important endophytic fungi in plants that form arbuscular mycorrhizas with roots. Arbuscular mycorrhizas can absorb mineral nutrients for plant partners through forming extensive networks of AMF hyphae in the soil in exchange of photosynthetically fixed carbon and lipids (Walder and van der Heijden 2015, Wu et al. 2019a). As a result, mycorrhizas promote plant growth under various abiotic and biotic stress conditions (Gąstoł and Domagała-Świątkiewicz 2015, He et al. 2019, Wu et al. 2019b, Zhang et al. 2019, 2020).

Plant roots have a high degree of phenotypic plasticity, and its morphology is affected by numerous factors, including soil microorganisms. Previous studies indicated that AMF affected the root morphology and photosynthetic rate of host plants (Heinonsalo et al. 2016). In trifoliate orange, inoculation with *Acaulospora scrobiculata* or *Funneliformis mosseae* could improve root morphological traits than non-inoculation (Wu et al. 2016). In tea plants, AMF remarkably improved total root length and volume, while decreased root hair length and number (Shao et al. 2018). However, AMF colonisation had no sig-

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nificant effects on root diameter or root hair length in a temperate forest (Eissenstat et al. 2015). It suggests that the mycorrhizal role in root morphology is dependent on AMF and hosts. Therefore, further studies need to be conducted to evaluate the combination of AMF and host plants. Another function of AMF is to help their hosts absorb nutrients from the soil, especially immobile elements such as phosphorus (P) (Li et al. 2019). Besides, a positive correlation was observed between P concentrations and root mycorrhizal colonisation in three bean varieties under glasshouse conditions (Ibijbijen et al. 1996).

Walnut (*Juglans regia* L.) is an important oil fruit tree and timber in the world. Earlier studies conducted by Ponder (1979) found that black walnut could be colonised by native AMF, and mycorrhizas had a positive response to walnut growth in old fields. Dolcet-Sanjuan et al. (1996) used two AMF species, *Glomus mosseae* and *G. intraradices*, inoculated into micropropagated *Juglans regia* clones Serr and MB-T-231 four weeks after acclimatisation. They noted that early AMF inoculation could improve post-acclimatisation growth and plant survival. In Ukraine, Kostenko et al. (2018) tried to explore the mycorrhizal colonisation status in walnuts of Ukrainian orchards and proposed the potential benefits of AMF on growth and pathogen resistance of walnuts. Melichar et al. (1986) also reported that AMF stimulated the growth of eastern black walnut seedlings, but the growth improvement under inoculation with *Glomus microcarpus*, *G. mosseeae*, and combination of *G. microcarpus* and *G. fasciculatus* was superior to under inoculation with *Glomus caledonius*. Xu and Tang (2013) found that native AMF colonisation of walnut was 32.41 ± 2.09%, and spore density was 11.0 ± 0.8 spores/10 g soil. Moreover, spore density in walnut rhizopshere was significantly and positively correlated with soil available P, available K, and cation exchange capacity, indicating the importance of AMF in soil nutrients. These results displayed that walnut trees depend on mycorrhizal symbiosis, while the information regarding the selection of dominant AMF species and nutrient responses to AMF is scarce. The present study was designed to evaluate the effects of five different AMF species on plant growth, root morphology, gas exchange, and nutrient acquisition of walnuts.

MATERIAL AND METHODS

Experimental design. This experiment was a completely randomised block design, consisting of six inoculations with *Acaulospora scrobiculata*, *Diversispora spurca*, *Glomus etunicatum*, *G. mosseae*, *G. versiforme*, and non-AMF. Each of the six treatments repeated six times, with a total of 36 pots.

Mycorrhizal fungus inoculums. Five AMF species, including *Acaulospora scrobiculata*, *Diversispora spurca*, *Glomus etunicatum*, *G. mosseae*, and *G. versiforme* were chosen, based on the results of Feng et al. (2005). These AMF strains used were provided by the Bank of Glomales in China (BGC, Beijing, China), and then propagated based on white clover as a host for three months under potted conditions. Mycorrhizal fungal inoculums consisted of spores, AMF extraradical hyphae, and AMF-colonised root pieces. The spore density of AMF was 15 to 18 spores/g in mycorrhizal inoculums.

Plant culture. Seeds of walnut (*Juglans regia* L. Liaohe 1) were provided by the Walnut Technology Promotion Center, Baokang, Hubei, China. Seeds were sterilised with 75% ethanol solutions for 10 min, rinsed six times with distilled water, and stored in autoclaved (0.11 MPa, 121 °C, 1.5 h) sand. Next year, stored seeds were germinated in a growth chamber with 28 °C/20 °C day/night temperature and relative humidity of 80%. A month later, two-leaf-old seedlings with the same size were transferred into plastic pots (15 cm in depth, 16 cm in mouth diameter, and 10 cm in bottom diameter) containing 2.1 kg of autoclaved soil and sand (3:1, *v*/*v*). Properties of the potting mixture used were pH of 6.2, 0.85% soil organic carbon content, and 14.6 mg/kg Olsen-P content. At transplanting, 100 g of inoculums from each fungus was applied into the rhizosphere of walnut. Non-AMF treatment received the same amount of autoclaved substrate (0.11 MPa, 121 °C, 1.5 h). Subsequently, all the inoculated pots were placed in a greenhouse from March 20 to June 20, 2019, where the photosynthetic photon flux density ranged from 550 to 900 μmol/m^2/s, 28 °C/20 °C day/night temperature, and 60–95% relative humidity.

Variable analysis. A sunny day before plant harvesting, leaf gas exchange including photosynthetic rates (P_n), stomatal conductivity (g_s), transpiration rates (E), leaf temperature, and intercellular CO_2 concentration (C_i) was determined using a Li-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, USA) from 9:30 am to 10:30 am. Plant height, stem diameter, leaf numbers, and total biomass were measured.

Root morphology was scanned with an Epson Perfection V700 Photo Dual Lens System (J221A, Jakarta Selatan, Indonesia) and analysed by the

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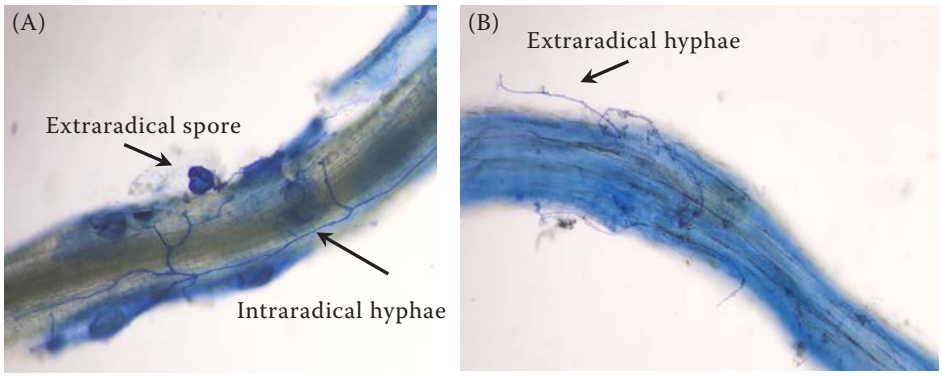

Figure 1. Root mycorrhizal colonisation of walnuts (*Juglans regia* L. Liaohe 1). (A) intraradical hyphae and extraradical spore; (B) extraradical and intraradical hyphae

WinRHIZO software (Regent Instruments Inc., Quebec, Canada) to obtain root length, projected area, surface area, volume, and average diameter. Similarly, the leaf area was analysed by the Epson Perfection V700 Photo Dual Lens System and the WinRHIZO. Part of 1-cm-long root segments was cleared with 10% (*w*/*v*) KOH and stained with 0.05% (*w*/*v*) trypan blue in lactophenol (Phillips and Hayman 1970). The mycorrhizal colonisation was quantified as the percentage of AMF-colonised root lengths *versus* observed root lengths.

Roots were oven-dried at 75 °C, ground into 0.5 mm powder, digested by H_2SO_4-H_2O_2, and measured by an electrochemical analyser (Smartchem 200, Scientific Instruments Limited, Weston, USA) for the analysis of N content and by an ICP Spectrometer (IRIS Advantage, Thermo, Waltham, USA) for the analysis of P, K, Ca, Mg, B, Cu, Fe, Mn, and Zn contents.

Statistical analysis. All the data were analysed with analysis of variation (SAS, v 8.1, Cary, USA), and significant differences between treatments were compared by Duncan's multiple range tests at $P = 0.05$ level. The percentage of root mycorrhizal colonisation was arcsine transformed before statistical analyses.

RESULTS AND DISCUSSION

Changes in root mycorrhizal colonisation of walnuts. Root mycorrhizas were not observed in the non-AMF-inoculated walnuts. In AMF-treated plants, mycorrhizal structures including internal hyphae, external hyphae, and vesicles were observed in roots (Figure 1), and root mycorrhizal colonisation varied from 47.0% to 76.4% (Table 1). There was no configurational change in the pattern of root mycorrhisation in different AMF species. Among the five AMF species, *D. spurca* showed the best root mycorrhizal colonisation, and *G. versiforme* represented the lowest mycorrhizal colonisation. Dixon (1988) reported higher root mycorrhizal colonisation by *G. deserticola* and *G. etunicatum* than by *Gigaspora margarita* in a black walnut source. Similarly, Melichar et al. (1986) also reported a variation in root AMF colonisation of eastern black walnut by *Glomus caledonius*,

Table 1. Effect of arbuscular mycorrhizal fungi (AMF) on root colonisation and plant growth of walnut (*Juglans regia*) seedlings

AMF treatments	Root AMF colonisation (%)	Plant height (mm)	Stem diameter (mm)	Leaf number per plant	Leaf area (cm^2)	Biomass (g/plant FW)
A. scrobiculata	54.97 ± 1.76^{c}	11.5 ± 0.9^{b}	6.74 ± 0.20^{bc}	7.50 ± 0.55^{b}	412 ± 26^{c}	31.84 ± 1.70^{c}
D. spurca	76.37 ± 6.70^{a}	14.3 ± 1.1^{a}	7.54 ± 0.58^{a}	6.83 ± 0.41^{c}	594 ± 24^{a}	42.70 ± 1.53^{a}
G. etunicatum	63.61 ± 5.53^{b}	14.2 ± 1.1^{a}	7.01 ± 0.53^{ab}	8.50 ± 0.55^{a}	518 ± 45^{b}	36.04 ± 2.21^{b}
G. mosseae	51.57 ± 0.97^{cd}	13.3 ± 1.1^{a}	7.30 ± 0.68^{ab}	5.67 ± 0.52^{d}	428 ± 38^{c}	32.55 ± 1.70^{c}
G. versiforme	46.99 ± 0.68^{d}	14.6 ± 1.3^{a}	6.14 ± 0.53^{cd}	6.00 ± 0.34^{d}	431 ± 38^{c}	37.14 ± 2.14^{b}
Non-AMF	0 ± 0^{e}	11.0 ± 0.9^{b}	5.61 ± 0.55^{d}	5.50 ± 0.55^{d}	315 ± 10^{d}	29.09 ± 2.09^{d}

Data (means ± standard deviation, $n = 6$) followed by different letters in the column indicate significant differences ($P < 0.05$). *A. scrobiculata* – *Acaulospora scrobiculata*; *D. spurca* – *Diversispora spurca*; FW – fresh weight; *G. etunicatum* – *Glomus etunicatum*; *G. mosseae* – *Glomus mosseae*; *G. versiforme* – *Glomus versiforme*

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Figure 2. Plant growth responses of walnut (*Juglans regia* L. Liaohe 1) seedlings inoculated with arbuscular mycorrhizal fungi (AMF)

G. microcarpus, *G. mosseeae*, and the combination of *G. microcarpus* and *G. fasciculatus*. Our results confirmed that walnuts could be colonised by AMF as mycorrhizal plants, and the root colonisation was highly dependent on AMF species.

Mycorrhizal effects on plant growth of walnuts. It is documented that AMF inoculation could improve plant growth of hosts, such as citrus, tea, hyacinth, etc. (Shao et al. 2018, Xie and Wu 2018). Our study indicated that plant height, stem diameter, leaf number, leaf area, and biomass of walnuts were increased, to some extent, by AMF inoculations (Table 1, Figure 2). Among the five AMF treatments, *D. spurca*, *G. etunicatum*, *G. mosseae*, and *G. versiforme* significantly increased plant height by 30.0, 29.1, 20.9, and 32.7%; *A. scrobiculata*, *D. spurca*, *G. etunicatum*, and *G. mosseae* significantly increased stem diameter by 20.1, 34.4, 25.0, and 30.1%; *A. scrobiculata*, *D. spurca*, and *G. etunicatum* notably increased leaf number per plant by 36.4, 24.2, and 54.5%; *A. scrobiculata*, *D. spurca*, *G. etunicatum*, *G. mosseae*, and *G. versiforme* considerably improved leaf area by 30.8, 88.6, 64.4, 35.9, and 36.8%; *A. scrobiculata*, *D. spurca*, *G. etunicatum*, *G. mosseae*, and *G. versiforme* considerably improved plant biomass by 9.5, 46.8, 23.9, 11.9, and 27.7% (Table 1). The varying growth responses in different AMF-treated walnut are due to the compatibility of AMF and host plants, as well as the change in AMF-modulated root morphology. As a whole, the relatively highest plant growth response was found in the *D. spurca*-inoculated walnuts (Table 1). This result is consistent with a previous study conducted by Dolcet-Sanjuan et al. (1996) and Carretero et al. (2009) in walnut trees. The growth improvement by mycorrhizal inoculation is often due to the root morphological modification, nutrient increases, and phytohormone regulation (Shao et al. 2018, Zhang et al. 2019).

Mycorrhizal effects on the root morphology of walnuts. Root morphology, the spatial morphol-

Table 2. Effect of arbuscular mycorrhizal fungi (AMF) on root morphological traits of walnut (*Juglans regia*) seedlings

AMF treatments	Length (cm)	Projected area (cm^2)	Surface area (cm^2)	Average diameter (mm)	Volume (cm^3)
A. scrobiculata	376.3 ± 33.8^{de}	45.17 ± 2.81^{c}	168.58 ± 11.44^{b}	1.25 ± 0.04^{a}	4.47 ± 0.33^{cd}
D. spurca	632.7 ± 38.7^{a}	64.45 ± 5.72^{a}	202.47 ± 17.97^{a}	1.27 ± 0.11^{a}	5.97 ± 0.42^{a}
G. etunicatum	552.1 ± 30.1^{b}	62.35 ± 3.47^{a}	161.53 ± 10.01^{b}	1.18 ± 0.07^{a}	5.09 ± 0.31^{b}
G. mosseae	421.4 ± 37.2^{cd}	51.42 ± 4.89^{b}	141.91 ± 8.82^{c}	1.27 ± 0.09^{a}	4.94 ± 0.40^{bc}
G. versiforme	464.6 ± 44.6^{c}	53.66 ± 4.99^{b}	195.87 ± 13.00^{a}	1.06 ± 0.06^{b}	5.17 ± 0.47^{b}
Non-AMF	366.4 ± 28.0^{e}	42.65 ± 3.90^{c}	134.00 ± 7.91^{c}	1.19 ± 0.07^{a}	4.11 ± 0.41^{d}

Data (means ± standard deviation, n = 6) followed by different letters in the column indicate significant differences ($P < 0.05$). *A. scrobiculata* – *Acaulospora scrobiculata*; *D. spurca* – *Diversispora spurca*; *G. etunicatum* – *Glomus etunicatum*; *G. mosseae* – *Glomus mosseae*; *G. versiforme* – *Glomus versiforme*

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ogy of the root system in the soil, can determine the ability of the plant to utilise soil resources and plays an important role in water and nutrient acquisition. In this study, root morphological traits of walnut seedlings varied among AMF treatments (Table 2). Compared to the non-AMF seedlings, a range of 2.7–72.7% (total root length), 5.9–51.1% (root projected area), 5.9–51.1% (root surface area), and 8.8–45.3% (root volume) were higher in the *D. spurca*, *G. etunicatum*, *A. scrobiculata*, *G. mosseae*, and *G. versiforme*-colonised seedlings than in the non-AMF-colonised seedlings, respectively. Hence, mycorrhizal walnuts showed better root morphology than non-mycorrhizal walnuts. The magnitude of root morphological alteration of walnut plants could be dependent on mycorrhizal fungal species. Our results were in agreement with previous studies on the grapevine and trifoliate orange (Aguín et al. 2004, Wu et al. 2019a). The alteration of root architecture under mycorrhisation may be derived from the results of improved root nutrition by AMF and from the changes in auxins and polyamines (Liu et al. 2018, Zhang et al. 2019, 2020). In addition, the average root diameter was not affected by AMF inoculation except for *G. versiforme*. Compared to the non-AMF treatment, *G. versiforme* inoculation significantly decreased root diameter in walnut seedlings by 10.9%, which is consistent with studies with woody plants of southern Brazil (Zangaro et al. 2007).

Mycorrhizal effects on leaf gas exchange of walnuts. In this study, leaf gas exchange of walnuts was affected by AMF inoculations (Table 3). Compared to the non-AMF treatment, leaf P_n, E, and g_s were respectively increased by 26.2, 36.6, and 66.9% with the inoculation of *D. spurca*, by 24.7, 40.3, and 66.2% with *G. mosseae*, by 16.3, 12.7, and 35.6% with *A. scrobiculata*, by 9.9, 11.2 and 42.1% with *G. etunicatum*, and by 6.8, 6.7, and 6.5% with *G. versiforme*. Among them, *G. versiforme* did not significantly change leaf P_n, E, and g_s, while other AMF species mostly improved leaf P_n, E, and g_s. Mycorrhizal walnuts recorded markedly lower C_i than non-mycorrhizal control, dependent on AMF species. Leaf temperature was significantly lower under inoculation with *D. spurca*, *G. etunicatum*, and *G. mosseae* than under non-AMF inoculation. Zhang et al. (2018) also observed that *Glomus mosseae* and *G. intraradices* significantly increased P_n, E, g_s, and C_i of castor bean. The study of de Araújo Diniz et al. (2010) showed that *Glomus clarum* had a positive effect on the leaf temperature of a young rubber tree. It implies that AMF improved the leaf gas exchange of walnuts, dependent on AMF species. In addition, the improvement of gas exchange by mycorrhisation is able to increase the C fixation during photosynthesis, which benefits for the growth of plants and AMF (Ding et al. 2020).

Mycorrhizal effects on root nutrient contents of walnut. As shown in Table 4, AMF inoculations altered root mineral nutrient contents of walnuts. Compared with non-AMF treatment, except for *A. scrobiculata*, the inoculations with *D. spurca*, *G. etuicatum*, *G. mosseae*, and *G. versiforme* significantly increased root N content by 35.6, 102.6, 24.9, and 20.2%, respectively. Hereinto, *G. etunicatum* exhibited the best-improving effect. It is known that mycorrhizal symbiosis stimulated N uptake and transport of host plants through mycorrhizal extraradical hyphae in the rhizosphere, and the

Table 3. Effect of mycorrhizal on photosynthesis of *Juglans regia* seedlings

AMF treatments	P_n (μmol/m^2/s)	E (mmol/m^2/s)	g_s (μmol/m^2/s)	C_i (μmol/mol)	Leaf temperature (°C)
A. scrobiculata	11.56 ± 0.45^{ab}	1.51 ± 0.10^{b}	60.21 ± 5.91^{b}	105.77 ± 8.28^{c}	35.72 ± 0.14^{ab}
D. spurca	12.54 ± 1.17^{a}	1.83 ± 0.15^{a}	74.13 ± 5.93^{a}	71.12 ± 6.73^{d}	34.45 ± 0.41^{c}
G. etunicatum	10.92 ± 0.73^{bc}	1.49 ± 0.08^{b}	63.15 ± 3.97^{b}	138.27 ± 12.99^{b}	35.32 ± 0.36^{b}
G. mosseae	12.40 ± 0.69^{a}	1.88 ± 0.11^{a}	73.81 ± 5.61^{a}	81.26 ± 7.38^{d}	32.53 ± 1.34^{d}
G. versiforme	10.62 ± 0.56^{bc}	1.43 ± 0.09^{bc}	47.33 ± 4.43^{c}	106.17 ± 6.71^{c}	35.82 ± 0.13^{ab}
Non-AMF	9.94 ± 0.96^{c}	1.34 ± 0.12^{c}	44.43 ± 4.33^{c}	161.36 ± 13.79^{a}	36.12 ± 0.16^{a}

Data (means ± standard deviation, n = 6) followed by different letters in the column indicate significant differences (P < 0.05). AMF – arbuscular mycorrhizal fungi; *A. scrobiculata* – *Acaulospora scrobiculata*; *D. spurca* – *Diversispora spurca*; *G. etunicatum* – *Glomus etunicatum*; *G. mosseae* – *Glomus mosseae*; *G. versiforme* – *Glomus versiforme*; P_n – photosynthetic rates; g_s – stomatal conductivity; E – transpiration rates; C_i – intercellular CO_2 concentration

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Table 4. Effect of arbuscular mycorrhizal fungi (AMF) on root mineral nutrient contents of walnut (*Juglans regia*) seedlings

AMF treatments	N	P	K	Mg	Ca	Fe	Cu	Mn	B	Zn
	(g/kg DW)						(mg/kg DW)			
A. scrobiculata	8.27 ± 0.71^{d}	0.89 ± 0.04^{c}	10.65 ± 0.66^{a}	1.29 ± 0.12^{e}	7.59 ± 0.27^{e}	0.83 ± 0.06^{d}	108.3 ± 4.76^{a}	51.31 ± 3.15^{d}	21.97 ± 1.58^{c}	42.39 ± 2.53^{cd}
D. spurca	10.62 ± 0.55^{b}	1.50 ± 0.08^{a}	10.24 ± 0.59^{a}	2.28 ± 0.11^{b}	7.85 ± 0.44^{de}	1.98 ± 0.18^{c}	110.69 ± 9.16^{a}	101.88 ± 8.21^{b}	21.46 ± 1.89^{c}	46.46 ± 2.41^{bc}
G. etunicatum	15.86 ± 0.86^{a}	1.31 ± 0.10^{b}	9.62 ± 0.59^{a}	1.72 ± 0.11^{d}	8.78 ± 0.63^{c}	1.88 ± 0.15^{c}	96.45 ± 6.99^{a}	98.08 ± 7.30^{b}	24.75 ± 1.74^{b}	41.33 ± 2.65^{d}
G. mosseae	9.78 ± 0.85^{bc}	1.30 ± 0.12^{b}	9.87 ± 0.44^{a}	2.52 ± 0.17^{a}	11.51 ± 0.24^{a}	2.41 ± 0.07^{b}	97.49 ± 8.57^{a}	102.83 ± 6.87^{b}	23.44 ± 1.19^{bc}	54.28 ± 4.99^{a}
G. versiforme	9.41 ± 0.67^{c}	0.90 ± 0.03^{c}	9.71 ± 0.80^{a}	2.16 ± 0.16^{bc}	10.62 ± 0.51^{b}	1.95 ± 0.18^{c}	97.65 ± 5.56^{a}	78.13 ± 7.76^{c}	25.37 ± 1.49^{b}	44.37 ± 3.13^{cd}
Non-AMF	7.83 ± 0.67^{d}	0.88 ± 0.04^{c}	9.52 ± 0.42^{a}	2.01 ± 0.16^{c}	8.29 ± 0.30^{cd}	2.82 ± 0.20^{a}	102.01 ± 9.06^{a}	143.97 ± 9.71^{a}	32.75 ± 2.18^{a}	50.91 ± 2.19^{ab}

Data (means ± standard deviation, n = 4) followed by different letters in the column indicate significant differences (P < 0.05). *A. scrobiculata* – *Acaulospora scrobiculata*; *D. spurca* – *Diversispora spurca*; DW – dry weight; *G. etunicatum* – *Glomus etunicatum*; *G. mosseae* – *Glomus mosseae*; *G. versiforme* – *Glomus versiforme*

contribution to N was reported up to 21–74% (Jin et al. 2005). Such a high contribution of AMF to N would stimulate the synthesis of amino acids and proteins, thereby raising the physiological activity of mycorrhizal plants.

In our study, *G. versiforme* and *A. scrobiculata* did not alter root P content, while *D. spurca*, *G. etuicatum*, and *G. mosseae* significantly increased root P content by 70.5, 48.9, and 47.7%, respectively (Table 4). AMF hyphae could penetrate into the soil around the roots of the plant so that the plant could get a larger volume of soil, resulting in higher uptake of nutrients from the soil. Root morphology alteration in AMF-colonised walnut could give the host plant access to more soils to explore more P (Mathur et al. 2018). In addition, the acid phosphatase of AMF could hydrolyse phospholipids, thereby stimulating the organic P mineralisation process (Li et al. 2019).

All the inoculations did not change root K and Cu content, while notably reduced root Mn and Fe content (Table 4). The activity of oxidising and reducing microorganisms in the soil determines the dynamics of Mn and Fe (Yang et al. 2013). One explanation regarding the reduction of root Mn and Fe content by mycorrhizal symbiosis is that mycorrhizas limited Mn and Fe uptake by increasing the activity of oxidizing microorganisms (Arines et al. 1992). Another explanation is that mycorrhizal colonisation decreased the number of Mn reducers and the release of Mn-solubilising root exudates (Posta et al. 1994). In this study, all the inoculations significantly reduced root B content (Table 4). The decrease in root B content may be connected with the "dilution effect" caused by the increase in biomass. AMF improved the potential of host plants to tolerate metal toxicity through the "dilution effect" mechanism (Baslam et al. 2011) or exclusive barriers of toxic elements (Cabral et al. 2015). The lower root Mn, Fe, and B content in response to mycorrhizal inoculation may be ascribed to the nutrient transferring from the root into leaf. Future studies should consider leaf and root nutrient changes together in response to mycorrhisation.

Compared with non-AMF treatment, *D. spurca* and *G. etuicatum* had no effect on root Ca content, *G. mosseae*, and *G. versiforme* significantly increased root Ca content by 38.9% and 28.2%, and *A. scrobiculata* dramatically reduced root Ca content (Table 4). In addition, *D. spurca* and *G. mosseae* observably increased root Mg content by 13.6% and 20.2%, while *A. scrobiculata* and *G. etuicatum* reduced it. Considering the "dilution effect", AMF treatments almostly increased the Ca/Mg ratio. The competition between Ca and Mg is a major limitation of plant mineral nutrient absorption (Magdziak et al. 2011). Our study also indicated that AMF decreased root Zn content except for *D. spurca* and *G. mosseae*. Nevertheless, previous studies found the increase of Zn in roots of hosts inoculated with AMF (Pfeiffer and Bloss 1988). This might be associated with the effects of different mycorrhizal fungi species, root phenotypes, host plants, and soil environments.

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The present study only considered changes in root nutrients in response to mycorrhisation. Futher work will analyse the whole change in leaf and root nutrient contents to reveal the mycorrhizal function on total plant nutrient acquisition.

In short, AMF inoculations could confer the positive effects on plant growth, root morphology, leaf gas exchange, and root nutrient acquisition of walnuts, dependent on AMF species. Hereinto, *D. spurca*, could be considered as the most effective mycorrhizal fungus in walnut.

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